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DWARF MISTLETOE SEED STORAGE BEST AT LOW TEMPERATURE AND HIGH RELATIVE HUMIDITY

by

Donald M. Knutson, *Plant Pathologist*

ABSTRACT

Dwarf mistletoe (*Arceuthobium campylopodum*) seed survival was determined for seed stored at 16 temperature x relative humidity regimes (-18°, 1°, 4°, 9°, C.; <2-, 6-, 10-, 75-percent relative humidity). Concentrated salt solutions (NaOH, $ZnCl_2$, and NaCl) and (anhydrous $CaSO_4$) were used for the different relative humidities. Germination was tested after 5, 10, and 15 months. The best germination was at 1° C. and 75-percent relative humidity: 94 percent (5 months), 80 percent (10 months), and 58 percent (15 months). Inoculations can now be done throughout the year, facilitating studies of the infection process and mass production of dwarf mistletoe-infected trees needed for outplanting studies.

Keywords: Dwarf mistletoe, *Arceuthobium campylopodum*, seed storage, seed germination.

Year-around inoculation of coniferous hosts with dwarf mistletoe (*Arceuthobium* spp.) seeds[1] requires a dependable method of seed storage. Heretofore, no satisfactory method of year-long storage has been reported for these short-lived seeds.

1/ The term "seed" is normally used, although members of the Loranthaceae have neither true ovules nor integuments (Kuijt 1960).

Several studies have emphasized the effects of temperature and relative humidity on seed viability and germination. Viability has been determined by the triphenyl tetrazolium chloride technique of Flemion and Poole (1948) as reported by Scharpf and Parmeter (1962) and Wicker (1962), and germination determined by placing surface-sterilized seeds on moist filter paper (Scharpf and Parmeter 1962) or in 1- to 5-percent hydrogen peroxide (Wicker 1962). Viability percent was generally higher than germination percent for *Arceuthobium abietinum* (Engelm.) Hawksw. & Wiens (Scharpf 1970), *A. laricis* (Piper) St. John, *A. campylopodum* Engelm., [2/] and *A. americanum* Nutt. ex Engelm. (Wicker 1965).

Low temperatures (1° to 5° C.) are best for storing dwarf mistletoe seeds 6 months or less (Beckman 1964, Beckman and Roth 1968, Knutson 1969, Wicker 1967). Viability was maintained longer at near freezing than at higher or lower temperatures for seeds of *A. occidentale* Engelm. [3/] (Scharpf and Parmeter 1962).

Seed storage experiments for longer periods have been reported. Beckman (1964) found 50- to 56-percent germination of *A. campylopodum* seed after 240 days' storage at 1.5° C. Fifty percent of *A. abietinum* seeds germinated after 200 days' storage at 2° C. (Scharpf 1970). After 12 months' storage at 0° to 5° C., 25 percent of *A. campylopodum* seeds germinated (Wicker 1965).

Relative humidities of 40 to 70 percent maintained *A. campylopodum* seed viability (55 to 65 percent) for 160 days at 1.5° C. Beckman 1964). Above 90-percent relative humidity, fungi destroyed the seeds. Reduced seed germination resulted from storage relative humidities of 75 percent or more when seeds were stored 3 months at 5° C. (Wicker 1965), and fungi destroyed all seeds at saturated relative humidity at 5° C. (Wicker 1967). For short-term storage (62 days at 15° C.) relative humidity had no effect on seed viability (Scharpf 1970).

[2/] Formerly *A. campylopodum* f. *abietinum*, *A. campylopodum* f. *laricis*, and *A. campylopodum* f. *campylopodum*, respectively. Nomenclature changes used in this paper are according to F. G. Hawksworth and D. Wiens. New taxa and nomenclatural changes in *Arceuthobium* (Viscaceae). Brittonia 22: 265-269, 1970.

[3/] Formerly *A. campylopodum* f. *campylopodum*.

Materials and Methods

Dwarf mistletoe seeds (*A. campylopodum*) were collected from *Pinus ponderosa* Laws. on the Deschutes National Forest, Oregon, in September 1968. Seeds were harvested by enclosing the mature fruits with a paper bag and shaking the limb to discharge the seeds onto the inner surface of the bag. The bags containing the seeds were placed immediately in a field icebox and stored later the same day at 1° to 2° C. and 100-percent relative humidity until allocated to storage treatments in December 1968.

Storage chambers consisted of 500-milliliter Erlenmeyer flasks with seeds placed on strips (100 per strip) of Scotch Brand Filament Tape.[4/] One strip was suspended in each of 16 flasks (fig. 1). Seeds were not surface sterilized or otherwise pretreated. Saturated solutions of NaOH, $ZnCl_2$, and NaCl maintained relative humidity at approximately 6, 10, and 75 percent, respectively. These salts were reported (Winston and Bates 1960) to maintain constant humidity over a wide range of temperatures. Drierite was used to provide relative humidity less than 2 percent. Storage temperatures were maintained at -18°, 1°, 4°, and 9° C. in laboratory refrigerators. Initially

Figure 1.--*Method of storing dwarf mistletoe seed at constant relative humidities.*

[4/] The use of proprietary or brand names does not imply approval by U.S. Department of Agriculture of the product to the exclusion of others which may also be suitable.

and after 5 and 15 months of storage, the relative humidities and temperatures were measured with an electric hygrometer and compared with values summarized by Winston and Bates (1960). Relative humidities in my experiments varied somewhat from values listed for the temperatures used in this study (table 1). Because of possible damage to the electric hygrometer, relative humidities and temperatures at -18° C. were not tested.

Both NaOH and $ZnCl_2$ are deliquescent, which could explain the lower relative humidities at higher temperatures (table 1). As the temperature is raised the amount of solute increases, so the normal situation of rising vapor pressure with rising temperature may be counteracted by a diminution of vapor pressure because of a higher concentration of solution.

Seed germination was determined initially and after 5, 10, and 15 months in storage. Twenty seeds from each of the 16 storage treatments were divided into two lots of 10 and each lot germinated in a petri dish in 15 milliliters of 5-percent H_2O_2 (Wicker 1962). Initial germination (77 percent) was used as a base percent and subsequent data expressed as a percent of this base.

Table 1.--*A comparison of expected and actual relative humidities over three concentrated salt solutions*

Value and temperature	Salt solution		
	NaOH	$ZnCl_2$	NaCl
	---- *Percent relative humidity* ----		
Expected[1/]:			
2, 5, 10° C.	6	10	75
Measured:			
1° C.	11	13	72
4° C.	7	13	74
9° C.	7	6	75

[1/] Winston and Bates 1960.

Results and Discussion

Longevity of dwarf mistletoe seeds was affected by both temperature and relative humidity (fig. 2). Seeds stored at -18° C. did not germinate although the seed appeared normal.

After 5 months' storage, average germination for all relative humidities declined with increasing temperatures (94 percent at 1° C., 65 percent at 4° C., and 60 percent at 9° C.). At 4° C. and 9° C., seed survived best at high relative humidities (fig. 2). In no treatment was the decrease in germination as dramatic as that shown by Beckman (1964) who found that less than 5 percent of the seeds survived after 160 days at relative humidities under 20 percent. Possibly, the sulfuric acid solutions he used to obtain different relative humidities were phytotoxic.

Germination after 10 months' storage at 75-percent relative humidity was 80, 65, and 58 percent for 1°, 4°, and 9° C., respectively. For other relative humidities only seeds stored at 1° C. germinated and, of these, less than 20 percent (fig. 2).

After 15 months' storage, 58-percent germination was obtained at 75-percent relative humidity and 1° C. The only other germination after 15 months' storage was at 75-percent relative humidity and 9° C.

Subsequent to this study, bulk seed batches have retained comparable germinability after 12 months' storage at 1° C. and 75-percent relative humidity. Molds have not been a problem in seeds stored at 75-percent relative humidity, as Beckman (1964) and Wicker (1965) reported them to be on dwarf mistletoe seeds stored at high relative humidities.

Viability of dwarf mistletoe seed can be maintained year-around, providing a source of inoculum for year-around production of infected trees for experimentation.

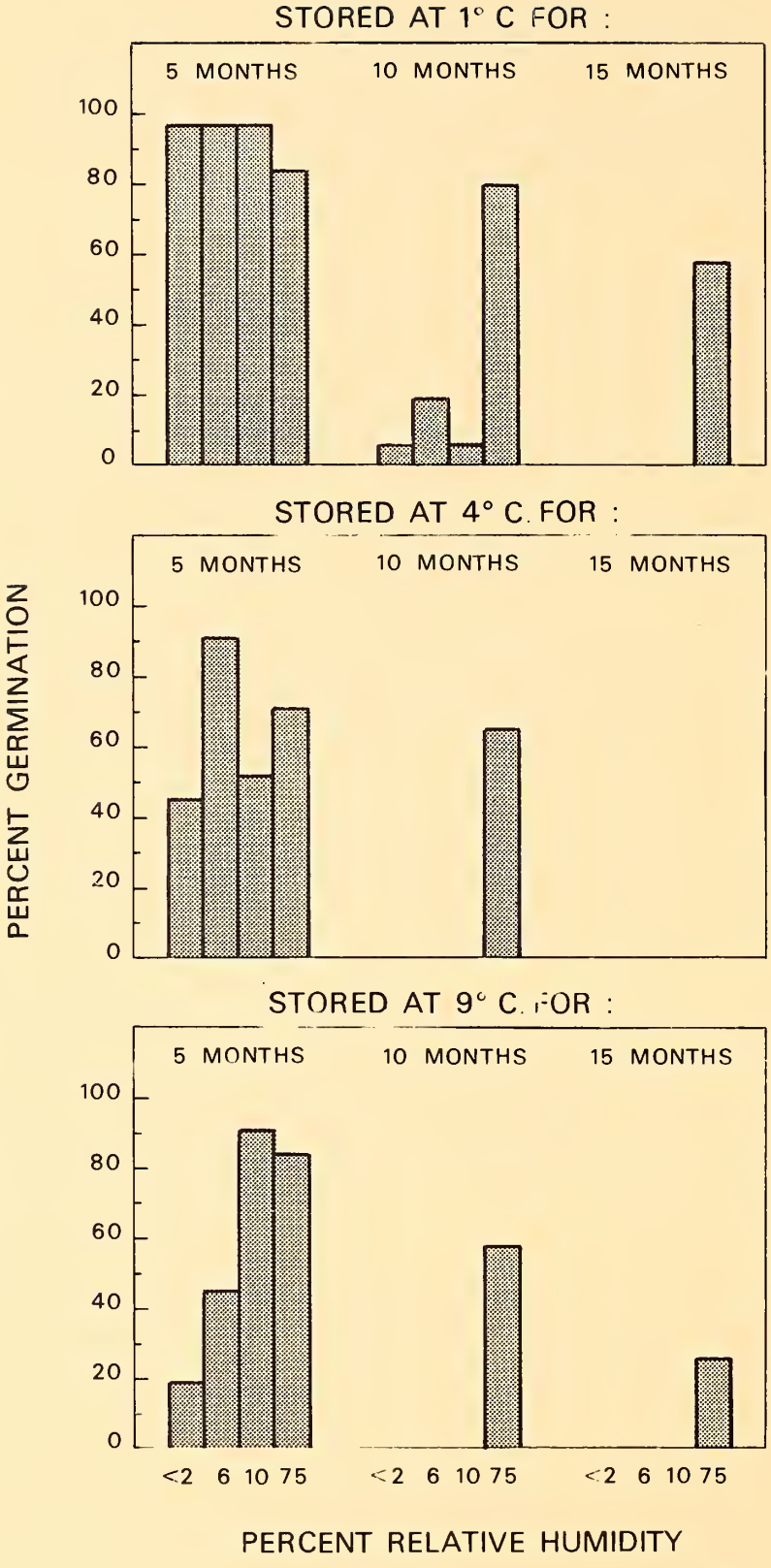

Figure 2.--*Germination percent of ponderosa pine dwarf mistletoe seed from 36 storage regimes (no seed germinated from the 18° C. storage regimes).*

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